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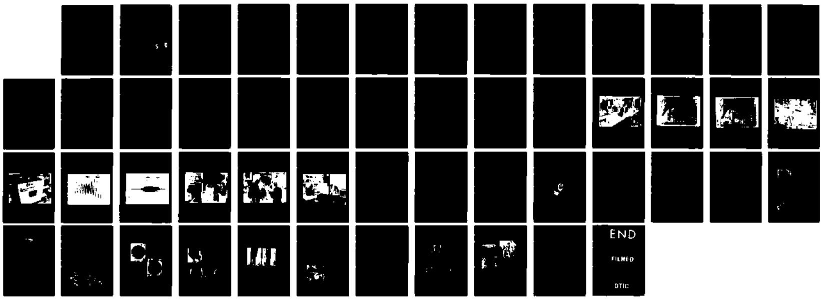

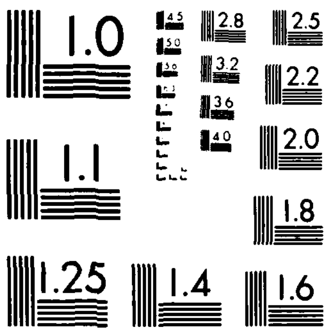

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REPORT DOCUMENTATION PAGE

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1. REPORT NUMBER	
2. GOVT ACCESSION NO.	
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle)	SUMMARY REPORT ON VISIT TO DEPARTAMENTO DE INVESTIGACION CENTRO RAMON Y CAJAL MADRID, SPAIN
5. TYPE OF REPORT & PERIOD COVERED	Final
6. PERFORMING ORG. REPORT NUMBER	ONR NR 665-037
7. AUTHOR(s)	R. A. Tell and E. Berman
8. CONTRACT OR GRANT NUMBER(s)	N00014-84-F-0167
9. PERFORMING ORGANIZATION NAME AND ADDRESS	U. S. Environmental Protection Agency 401 M Street, SW Washington, DC 20460
10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	61153N41 RR04101 RR041010A
11. CONTROLLING OFFICE NAME AND ADDRESS	Office of Naval Research (Code 441CB) 800 N. Quincy Street Arlington, VA 22217-5000
12. REPORT DATE	30 Oct 84
13. NUMBER OF PAGES	50
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	
15. SECURITY CLASS. (of this report)	U
15a. DECLASSIFICATION/DOWNGRADING SCHEDULE	

16. DISTRIBUTION STATEMENT (of this Report)

Unlimited

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

Unlimited

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Electromagnetic Fields, Pulsed Magnetic Fields, Chicken Embryo

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

R. Tell and E. Berman visited the laboratory of Dr. J. Leal in Madrid, Spain for the purpose of learning and appraising the experimental conditions used in Leal's studies of chick embryos in low-level magnetic fields. Extensive discussions were held during this time about all aspects of the methodology employed, who accomplished each aspect of the methods, and what were the goals of the experiments, as well as the conduct of extensive measurements and observations of the experimental conditions and local milieu using equipment brought specifically

Summary Report on Visit to Departamento de Investigacion
Centro Ramon y Cajal
Madrid, Spain

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Office of Radiation Programs

and

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October 30, 1984

U.S. Environmental Protection Agency

SUMMARY REPORT ON VISIT TO DELGADO LABORATORY, MADRID SPAIN

Background

During September 7-15, 1984, Dr. Ezra Berman and Mr. Richard A. Tell traveled to Madrid, Spain to visit with Professor Jose M. R. Delgado, Director of the Departamento de Investigacion, Centro Ramon y Cajal, and his colleagues for the purposes of discussion, observation, and characterization of experimental methodology used in their research on the effects of pulsed magnetic fields on the chick embryo. This memorandum documents our trip to Madrid and is intended to meet the requirements of a report of our findings in accordance with Navy Delivery Order number N00014-84-F-0167, dated July 18, 1984 (see Attachment A).

This trip was stimulated by the publication of two papers: (1) Delgado, J.M.R. et al., Embryological changes induced by weak, extremely low frequency electromagnetic fields, J. Anatomy (1982), 134, 3, pp. 533-551; (2) Ubeda, A., et al., Pulse shape of magnetic fields influences chick embryogenesis, J. Anatomy (1983), 137, 3, pp. 513-536. These two papers reported significant effects on chick embryonic development, apparently caused by very weak (in the range of approximately 0.1 - 12 micro Tesla) magnetic fields pulsed at rates between 10 and 1,000 pulses per second. This trip was arranged because of the potential health significance of the reported phenomenon, the possibility for new explorations of the underlying biophysical mechanisms of interaction of electromagnetic fields, the relatively sparse description of the engineering parameters of the experimental protocols in the published

papers, and the inability of others to repeat the findings of the two papers.

The direct travel expenses associated with both travelers were paid by the Office of Naval Research through the above Navy Delivery Order. No compensation for salaries was received through this arrangement with the Navy. The actual laboratory visit took place during September 10-14, allowing us five full days to interact with the personnel in the laboratory, to hold detailed discussions, and to make physical measurements on the experimental apparatus. During our visit, Dr. Thomas C. Rozzell with the Office of Naval Research Branch Office in London was also present and participated in our discussions and observations.

During our visit, we first met with Professor Delgado and received an orientation to the laboratory facility within which the magnetic field work is being performed. In addition to our detailed interaction with Dr. Jocelyne Leal and her colleagues on the subject of this report, we also had the opportunity to visit with Professor F. Rubia who is responsible for all of the research accomplished at the Centro Ramon y Cajal. During this visit with Professor Rubia, we were able to convey the potential importance of the embryo work and he seemed impressed as to the studies' potential significance from a public health point of view.

On Tuesday, September 13, Dr. Berman and Mr. Tell were allowed to present seminars on our own work to a group of interested department researchers. In the morning, R. Tell gave a presentation on work within

the Office of Radiation Programs to repeat the embryological findings. Later, in the afternoon, Dr. Berman presented material from his own work on the effects of microwave exposure on rats and mice. R. Tell then gave a presentation on the environmental assessment part of the nonionizing radiation program within EPA.

Laboratory Tour

The Departamento de Investigacion is conducting a broad range of research involving magnetic fields. The laboratories occupy areas of three different floors within the department. The attached floor plans (Attachment B) illustrate the laboratory layout. It appears that there are approximately 20-25 individuals directly involved in some sort of investigation of the effects of magnetic fields on biological systems. The laboratories are housed in a large 1,600 bed hospital building which is approximately eight years old. We were told that the department includes approximately 100 people, half of which are scientists and the remainder technical support personnel. The disciplines include immunology, behavior, cell biology, embryology, microbiology, and neural chemistry. The facilities have a new appearance and are well kept. Relatively modern equipment was in evidence in most laboratories. A central computer group provides support to the department although the main computer is somewhat antiquated by comparison with present-day technology. Delgado indicated that there was a move afoot to go toward end-user computer resources. There are apparently three small computers now being used within some of the programs of the department.

The animal facilities are adequate, clean, and well equipped. Two "rules" are violated: species separation and open windows. These are examples of facility limits, and are not due to carelessness or lack of knowledge.

Professor Delgado gave us a copy of a pamphlet titled Magnetic Fields in Biology (see Attachment C) which overviews some of the work being performed within the various laboratories. He was particularly excited about studies using Lactobacillus bacteria and the work involving Drosophila melanogaster which has reportedly shown a heritable mutagenic effect from pulsed magnetic fields (Ramirez, et al., Bioelectromagnetics 4:315-325, 1983), and the work on the so-called dialytrode (Delgado, et al., J. Neurochem. 42: 1218-1288, 1984). He seemed driven to find a model system which would produce a statistically measureable biological response in the shortest possible time. This seemed to be the reason for his apparent present high level of interest in the bacterial studies. Professor Delgado indicated a need for a recording polarimeter to further some studies in which magnetic fields change the polarization of light transmitted through amino acids (Faraday effect). Time did not permit obtaining technical details on most of the research work he discussed.

In summary, it appears that a large effort is being put into the study of the possible biological effects of magnetic fields. The costs associated with the exposure equipment are minimal and the personnel are able to apply their own professional backgrounds and research equipment to the problem making the overall effort an apparently cost-effective approach to doing some very interesting biological effects investigation.

The amazing thing is the large number of essentially diverse studies which all seem to show effects induced by exposure of biological systems to relatively weak, magnetic fields. It was our observation that most of the investigators tended to be independent of one another; i.e., it was not evident that there was a significant degree of interaction between the various research projects leading to a sense of isolation between the researchers.

Leal's Laboratory

The primary focus of our visit was the work on chick embryos described above. This study is performed by a small group of people, assembled about 4 years ago to perform the initial experimentation seen in the 1982 papers by Delgado, et al and Ubeda et al. The group has been continuously active and, besides the 1982 studies, has performed a number of studies leading to a characterization of the biological response of their model . . . the young chick embryo.

The laboratory contains three full-time people:

1. Jocelyn Leal, a cell biologist trained with a Ph.D. in Paris, heads the group.

2. Alejandro Ubeda, an M.D., and a Ph.D. candidate nearing the completion of his biological thesis; and

3. Angeles Trillo Ruiz, wife of Ubeda; also on the same career track, but somewhat later; has about 7 years experience in chick embryo studies and in glycosaminoglycans studies.

Above Leal is Professor Delgado, who acts more in an administrative capacity than a scientific manager. Leal is the driving force behind the scientific philosophy and discipline used in their studies on chick embryos.

The laboratory has been engaged in an examination of the effects of very low intensity pulsed magnetic fields on the 48-96 hour domestic chick embryo. Since the appearance of the publications of 1982, the group has done additional studies involving the effect of such fields on chick embryo:

1. orientation, alone (see URSI 1984 abstract, attached)
2. morphologic responses at various orientations
3. mitotic indices in neural groove tissue
4. effects in 50 Hz magnetic fields, and
5. the magnification of the effect after the end of exposure.

This experimental load has been a large effort for this small group. All exposures, biological preparations, and observations are conducted by the group without additional technical help. The methods used by the group are time-consuming and exacting, and are conducted in a "blind" fashion.

The biological investigations are conceptually valid. The work is directed to the examination of chick embryos using techniques which form corroborative analyses: morphological evaluation of the embryo as to stage of development; morphological evaluation as to the normality of the embryo; histological examination of select embryos for normal cell and extracellular structure, and the incidence of mitotic structures in embryos. All are aspects of the developmental regularity of the embryo and are expected to confirm each other to a significant degree.

The group voiced their concern about their inability to control various biological aspects of their studies. In particular, the origins and status of eggs are not yet controllable (see below for details), and may be indeterminative. No ready solution to this important factor is apparent, so that high uncontrolled variability (15-30 percent abnormals in controls) may remain in their studies for some time. Other sources of variability, such as paired but not identical conditions of incubation for control vs. treated samples, may yield to correction more readily by the use of new appropriate equipment.

The *in ovo* laboratory work is carried out in Room 2075, approximately 15 feet by 28 feet with the long axis of the room oriented in a north-south direction. The south end of the room faces to the outside and consists of large windows covered with venetian blinds. Laboratory benches line the entire east side of the room and it is here that most of the experimental work is done. Figure 1 is a photograph of the laboratory. Fixation, embedding, sectioning and staining of histological serial sections of embryos are done in another laboratory by this same group.

Eggs are procured from a supply house that caters to the research community. Unfortunately, it is impossible for Leal to determine the actual farm from which the eggs are obtained by the supplier, even though she has tried on several occasions. She was informed that the farm supplying eggs used in her earlier work had gone out of business some time ago. Now it seems as though the eggs may come from several farms before being pooled and provided to the hospital. Discussion of this topic seemed to indicate that Leal felt it was impossible for her to know the precise origin of the eggs, although the eggs are called "fresh", meaning that they are "less than 24 hours old."

Leal's concerns about the control of the source (read, quality) of eggs are extremely important and very much deserve an adequate solution. The characteristics of development that are being measured by Leal are very related to the hen and flock laying the eggs (genetics, nutrition, husbandry), to the care of the eggs from the time of laying to the time of delivery to Leal's group, and to seasonal adjustments in the laying hens. Without control or, at the very least, a description . . . of the characteristics of the source of the biological model under observation, it will not be clear that the model operates within conventional limits. Leal's experimental comparisons are usually kept to intra-experimental comparisons, and do not usually rely on comparisons of the results of one study with those of another. This methodology ignores what may be a high level of abnormal classifications in one experimental control group and a low level in another, and limits the investigator's interpretation to within each study. While not being sure that attempts to solve this important biological consideration will lead to lower variation than

already experienced, we feel that a solution is needed. We were told that there was no possibility that the animal facility could be used to supply eggs from hens maintained in the facility.

Normally 5 dozen eggs are obtained at one time. After an approximate 20 minute car or van ride, depending on the occasion, the eggs are brought into the laboratory and placed in a refrigerator in the laboratory room at 10° C within 1 to 3 hours after arrival. The eggs remain in the refrigerator at least 8 hours before use. They remain in the refrigerator in a vertical position. At most, the eggs remain up to 5 days prior to use but never more. The eggs are used at a fairly sonable rate which means that all or nearly all of the 5 dozen supply is exhausted by the end of a 5-day week.

Prior to the beginning of an experiment, the required number of eggs are taken from the refrigerator and placed into flat wooden egg holders, usually with the small end of the egg to the south but not always, on a flat work table in the center of the room. An 'X' is written on the top side of each egg along with a serial number using a graphite pencil. The eggs are then allowed to remain on the benchtop in this horizontal position overnight before initiation of the treatment. When the experiment is to begin, eggs are alternately assigned to treatment and control groups. But the horizontal position of the egg is never changed; during the treatment or control condition and their subsequent removal from the incubator and opening for observation of the embryo, the 'X' remains facing upwards.

The historical description of the experimental procedures is complex. In the early experiments, exposures were conducted using 2 solenoidal coils connected in series to a Grass Instruments Model S-88 pulse generator in a single incubator in another laboratory room in the hospital. Controls were simply placed adjacent to the coils within the incubator. More recently, and for what seemed to be for most of the work accomplished so far, 2 and sometimes 5 coils are used in series with either a Grass Instruments Model SD-9 or S-44 pulse generator. Also, a square Helmholtz coil, 30 cm on a side with a coil spacing of 19 cm, connected to the SD-9 has been used. Thus, a range of pulse generators and two kinds of coil exposure systems have been used in the course of the work. The solenoidal coils are approximately 17 cm in length and have a diameter of about 7 cm. Each coil consists of 1,000 turns of 0.33 mm diameter enamelled copper wire. Physical examination of about 7 such coils showed that windings were not precisely uniform from coil to coil. In some cases the windings were loose enough to be moveable on the coil form.

Experiments are performed by placing the eggs to be treated in one incubator within either solenoidal coils or the Helmholtz coil apparatus and the controls in another incubator on the other side of the room. The control eggs are <u>not</u> placed within coils as are the treated eggs. Two Memmert incubators are employed: the larger one is a model TV40U, serial 559281 rated at 2,200 watts and designed for 220 volt AC operation. The smaller one is a model TV30B, serial 683140 rated at 800 watts and also designed for 220 volt AC operation. These are electrically heated units using a two stage heating element system; for large temperature changes

within the incubator, more current is used than when some smaller temperature differential exists. They were not true proportional systems in which heating current is proportional to the difference between actual temperature and desired set-point temperature. The larger unit's interior dimensions measured 60 cm wide, 58 cm tall, and 40 cm deep. Ribs presumably containing the electrical heating elements were approximately 1 cm thick extending from the interior surface. When the Helmholtz coil is used, it is placed such that its center is 29.5 cm from the bottom of the interior of the incubator. The small incubator measured 39.5 cm wide, 39.5 cm high, and 33.0 cm deep on the interior. The small unit had an internal glass door to protect against thermal shocks when opening the main door while the large incubator was not so equipped.

It is not clear what is the long term temperature stability of the two incubators. Clinical thermometers are used to measure the interior temperatures. No continuous monitoring is accomplished. Discussions with Jose Luis Monteagudo, the principal engineer in the department, indicated a long-term stability of $\pm 1^\circ$ C without opening the doors of the incubators. Conduct of an experimental run beginning on Wednesday, September 12, showed the following temperatures obtained by use of Yellow Springs thermistor probes placed in both the treatment and control incubators. The doors were closed at 12:00 noon on Wednesday and not reopened until Friday at noon.

RECORD OF INCUBATOR TEMPERATURES

Day	Hour	Indicated Temperature (°C) Treated	Control
Wednesday	12:00	34.2	36.0
	12:10	35.0	36.4
	12:25	34.8	36.5
	13:19	35.8	37.4
	15:00	37.8	38.0
	16:10	37.5	38.6
	16:52	37.5	38.3
	18:15	37.6	38.6
Thursday	09:45	37.5	38.4
	11:45	37.6	38.7
	14:45	37.8	38.2
	15:57	37.6	38.2
	16:15	37.1	38.6
	17:00	37.0	38.2
Friday	09:30	37.6	38.3

These data show as much as a 3.6° C variation in the treatment incubator and a 2.7° C variation in the control incubator. Differences in temperatures between the two incubators may be partly due to the fact that

we had made adjustments to the treatment incubator on Tuesday and it may not have been readjusted to the degree of precision that Ubeda normally exercises. The temperature variations appear to substantially stabilize after the first day. Some of this temperature variation will always occur because of the time the door is kept open to insert the eggs and to check temperatures using the mercury thermometers.

Humidity is controlled by the placement of two small containers of water in the bottom of the incubators. Due to an instrument malfunction, it was not possible to measure the relative humidity of the incubators but it appeared that the humidity might be relatively low since air was not blowing over the small open dishes. The researchers do not measure the relative humidity nor know if there is a difference between the two incubators.

It should be noted that the waveform of the various pulsed fields used varies somewhat with the intensity of the field being used. That is the loading on the generator was observed to affect the nature of the waveform. Figures 2 and 3 illustrate the difference in the waveform when the magnetic field amplitude is changed from 10 μT (Figure 2) to 100 μT (Figure 3). There is a noticeable change in the negative overshoot of the pulse. Alejandro Ubeda pointed out that all of the magnetic field intensities are specified in terms of the peak-to-peak value of the pulse. The coils are excited with the pulse from one of the generators connected in series with a capacitor and resistor. The magnetic field intensity is determined by adjusting the voltage drop across the resistor until it implies that the correct current is flowing in the coils to

create the desired field intensity. Figure 4 shows Alejandro Ubeda adjusting the circuit using an older, tube-type Tektronix oscilloscope.

Discussions with Jose Luis Monteagudo regarding the experimental set-up revealed that he had employed a 1 MHz low pass filter in preparing the oscilloscope photographs of the pulse waveforms. These photographs, which were smoothed by the filter for appearances sake, were then apparently traced for the figures appearing in the published article. No filter is used during actual excitation of the exposure coils. During the visit pulse waveforms generated only by the SD-9 pulse generator were observed (Figures 2 and 3) and it was apparent that the pulse did have some noise content which is presumably why the filter was used to smooth the apparent waveform for presentation purposes.

Measurements were made of ambient 50 Hz electric (E) and magnetic (B) fields in the laboratory and in the larger of the two incubators. Typical B field values were in the range of 1.0 - 1.5 mG within the lab and E fields were in the 1-4 V/m range, except near electrically connected equipment. Very much higher E field strengths were found near the two incubators. Measurements with a voltmeter showed that a potential of approximately 100 volts existed on the case of the large (exposure) incubator with respect to a grounded electrical conduit. This potential apparently exists because of capacitive coupling between the electrical heating elements and the case. The voltage did not present a shock hazard; i.e., it represented a very high source impedance. Fields of approximately 100 V/m could be found within 30 cm of the surface of the incubator. It was noted that the refrigerator in the laboratory did not

exhibit the enhanced fields near its surface. Subsequent investigation showed that the refrigerator used a grounded plug which we found to be unusual and not like other equipment we found in the laboratory. Internal 50 Hz B and E fields were measured to be 67-78 mG and 3.5 V/m respectively at the center of the large incubator.

Subsequent voltmeter readings showed that 55 volts potential difference existed between the output terminals of the pulse generator and the case of the incubator. This means that 50 Hz E fields interior to the incubator will be high depending upon the coil conductor to incubator case spacing. This may or may not have implications for the effects being found in the embryos. It was not possible to measure, with the equipment we had, the 50 Hz B and E fields inside of the solenoidal coils. It is conceivable that due to the tight windings on the solenoidal coils a shielding effect might exist for the eggs. Since control eggs are not placed within coils in the control incubator, they may be subject to higher 50 Hz electric field internal to the incubator than are the treated eggs.

Mechanical vibration of the incubators was determined by using a seismographic-quality accelerometer and measuring the voltage output on an oscilloscope. Mesurements were first made with the accelerometer placed in the center of the top of the incubators. Figure 5 shows this measurement setup. Figures 6 and 7 show the detected output of the accelerometer for the larger and smaller incubators respectively. These scope photographs show that the peak-to-peak amplitude of mechanical vibrations were approximately 10 times larger in the large incubator

(which is the exposure incubator). The calibration of the accelerometer is such that 8 volts of output is equivalent to 1 unit of gravitational force. Thus the large incubator exhibited a vibration of about 0.0188 g units peak-to-peak while the small unit had a vibration of only 0.00188 g units peak-to-peak. Subsequent internal vibration measurements showed that the magnitude of the vibratory motion inside of the incubator was equal in magnitude to the exterior measurements. A test measurement determined that there was no difference in these vibrations with the power to the incubator turned off or with the electric power cord removed completely from the wall receptacle. It was noticed throughout our visit that a very high air flow rate existed in many of the laboratories of the department. This is a possible source for the vibration. The smaller amplitude vibrations of the small incubator are probably due to its smaller physical size and the fact that a heavy piece of equipment was placed upon the top (see Figure 8).

Measurements were also made of the DC magnetic field of the earth. In Leal's laboratory a value of 0.34 Gauss was found throughout most of the room. Values of 0.33 to 0.48 G were found inside the large incubator depending upon exact location. Additional magnetic field measurements were made in the front part of Leal's office (0.33 G), in the hallway near the laboratory (0.41), and inside Room-2011D which is a large shielded room (0.33 G). Measurements of 50 Hz B and E fields in the shielded room showed values of about 0.01 mG and 2-4 V/m respectively. Thus, the shielding seemed to not have a significant effect on reducing ambient 50 Hz E fields within the department but did exhibit a substantial reduction to the B fields, i.e., a reduction factor of about 10-20 times.

Summary and Recommendations

On the behest of ONR, R. Tell and E. Berman visited the laboratory of Dr. J. Leal in Madrid, Spain during a period of 5 days, for the purpose of learning and appraising the experimental conditions used in Leal's studies of chick embryos in low-level magnetic fields. Extensive discussions were held during this time about all aspects of the methodology employed, who accomplished each aspect of the methods, and what were the goals of the experiments, as well as the conduct of extensive measurements and observations of the experimental conditions and local milieu using equipment brought specifically for this reason.

Leal's laboratory contains herself and two professional biologists, and is a small part of a large research unit (Departamento de Investigacion) attached to a large and modern hospital (Centrol Ramon y Cajal). The research unit specializes in investigations of the clinical applications of magnetic fields, particularly in their usefulness in emotional control and bone healing, but a wide variety of experiments using other biological and physical disciplines are also conducted. The Departamento is housed on three floors of an annex to the clinical buildings; except for the animal facility, the available space is uncrowded. Equipment in evidence appears to be generally adequate, if somewhat less than current. Complete freedom of the laboratory was available.

The discussions with Leal and her co-workers were full and completely open. Within the limits of foreign language-related communication,

discussions were rapid, comprehensive, and frank enchanges were common (See Figures 9 and 10). It is our feeling that no aspects of the work being carried out were hidden or obfuscated. Instead, new and unpublished results were freely shown for discussion. It is also our feeling that part of the eagerness with which our interest was sought was a reflection of the isolation from the outside world that seems now to be dissipating.

The biological manipulations and observational techniques appear to be conventional and sound, and, except for specific logistic and equipment problems, are fully controlled. The work is thorough in that checks are built into the system of observations where possible. However, at certain places in the examinations of biological materials, evaluations are required which may introduce trends into the data. But all studies are run blind and should remove most special tendencies for grading treated groups differently than control groups.

Control groups and treated groups are experimentally maintained in different conditions, so that 'controls' do not assume 'sham' status. Though the investigators appear to have checked for biological differences associated with different incubation conditions, and have not been able to identify any, they still exist and cannot be ignored when experimental comparisons are finally made.

Physical factors exist in the general laboratory, in Leal's laboratory, and in specific equipment used in the studies that may be important influences on the outcome of the experiment and may be so confounding and unique that the work may not be universally applicable.

The group was unaware of these factors because the measurements require equipment not available in the laboratory and such measurements had not been attempted before. The control conditions experience these factors to a different degree than the treated situation, and this fact aroused particular concern. Like the difference seen with control and treated eggs in their incubation, the different physical environmental may be contributory to the end result.

Because of the measurements made in the laboratory, a clearer description of the equipment used in the experiments can now be made. The characteristics of pulse generation equipment were described, and the actual pulse more clearly explained. Also, the idiosyncracies of the circuits used for reasons other than experimental were described. These aspects, like those described in the two paragraphs above, may define the exposure system as being so unique that its duplication in other laboratories may not be possible. Aside from these observations and based on our visit and collective judgements, we can not see any particular reason why the reported findings from this laboratory should not be able to be reproduced elsewhere.

Recommendations for further investigations assume that the last statement is true. Instead of encouraging duplication of the system now in use, we suggest that funds be used to further two directions of experimentation: 1) setting up a study in which experiments are conducted by Leal using new, more appropriate equipment, and which is duplicated in this country at the same time, and 2) upgrading Leal's capabilities by relieving the group of time-intensive techniques which can be accomplished

by others (have histological techniques done by others), include skills (electrical engineering) now not easily available, and fund specific equipment purchases (temperature and humidity monitoring capability in addition to perhaps the incubation system itself) to ensure full control of incubation procedures. A third recommendation has already been emphasized to Leal: that of never disassembling the experimental apparatus now in use.

Figure 1. Photograph of Dr. J. Leal's laboratory. The windows face to the south.

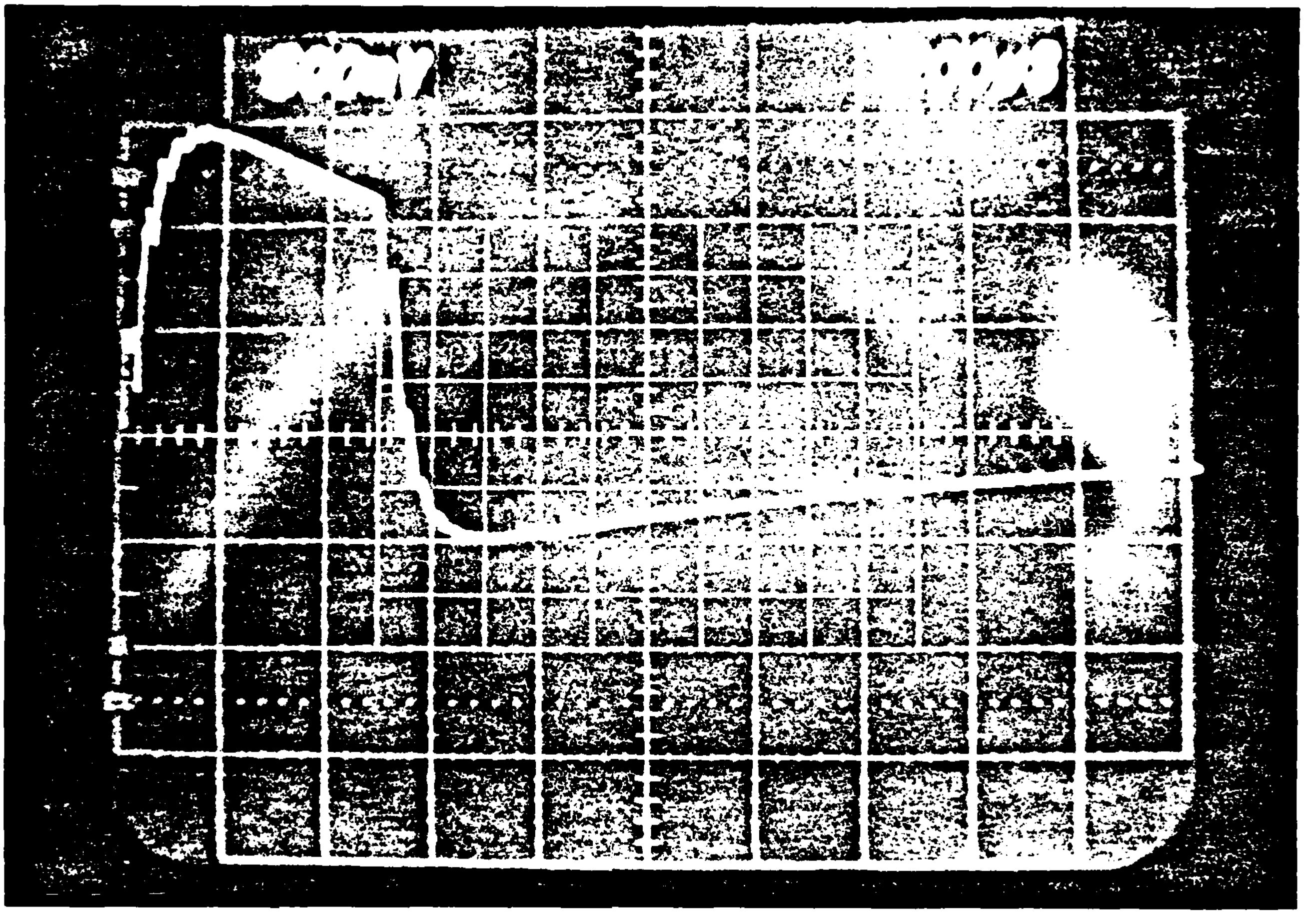


Figure 2. Oscilloscope photograph of waveform of pulse from Grass Instruments Model SD-9 adjusted for a 10 μT field when connected to 5 solenoidal coils in series.

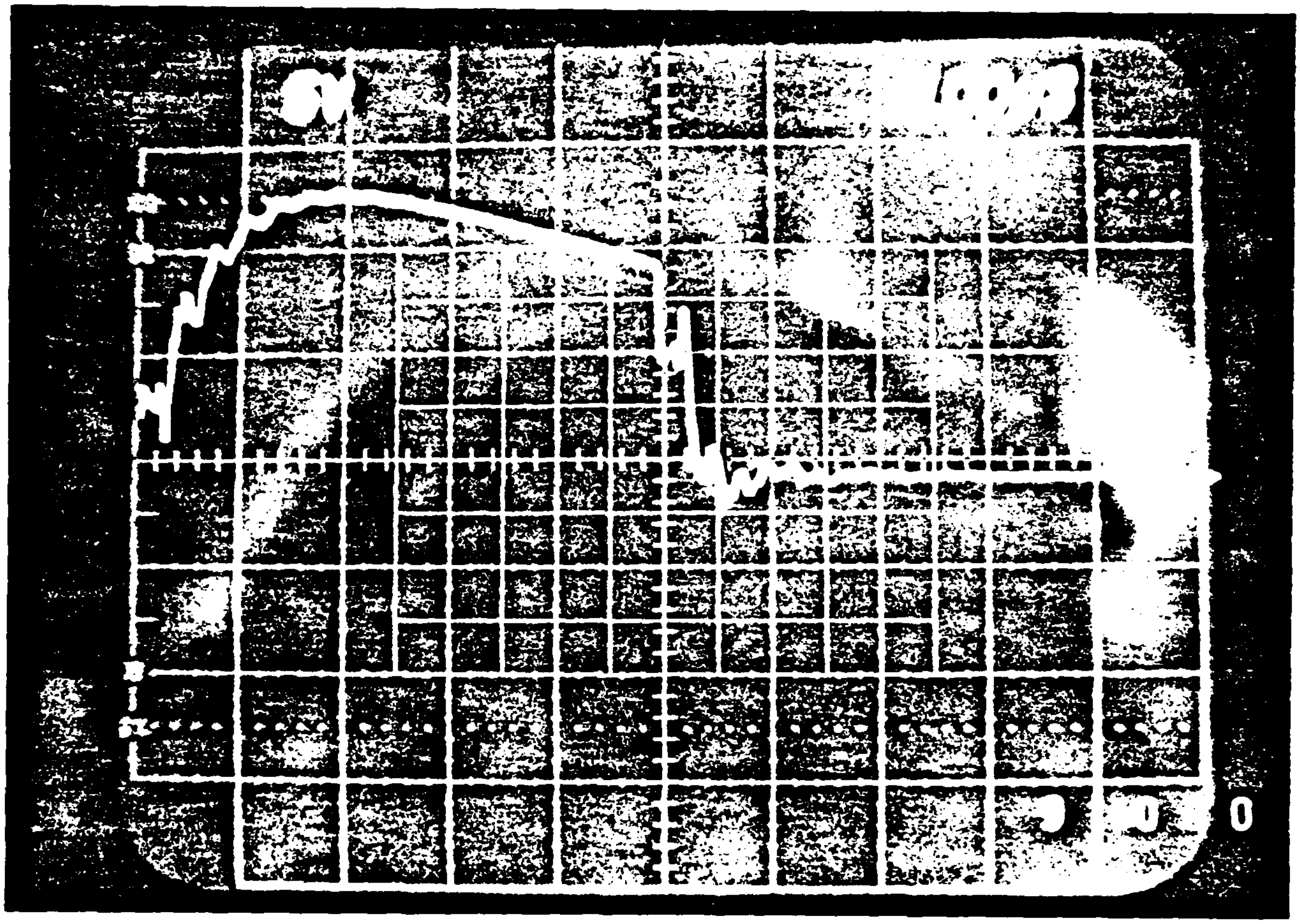


Figure 3. Oscilloscope photograph of waveform of pulse from Grass Instruments model SD-9 adjusted for a 100 μT field when connected to 5 solenoidal coils in series.

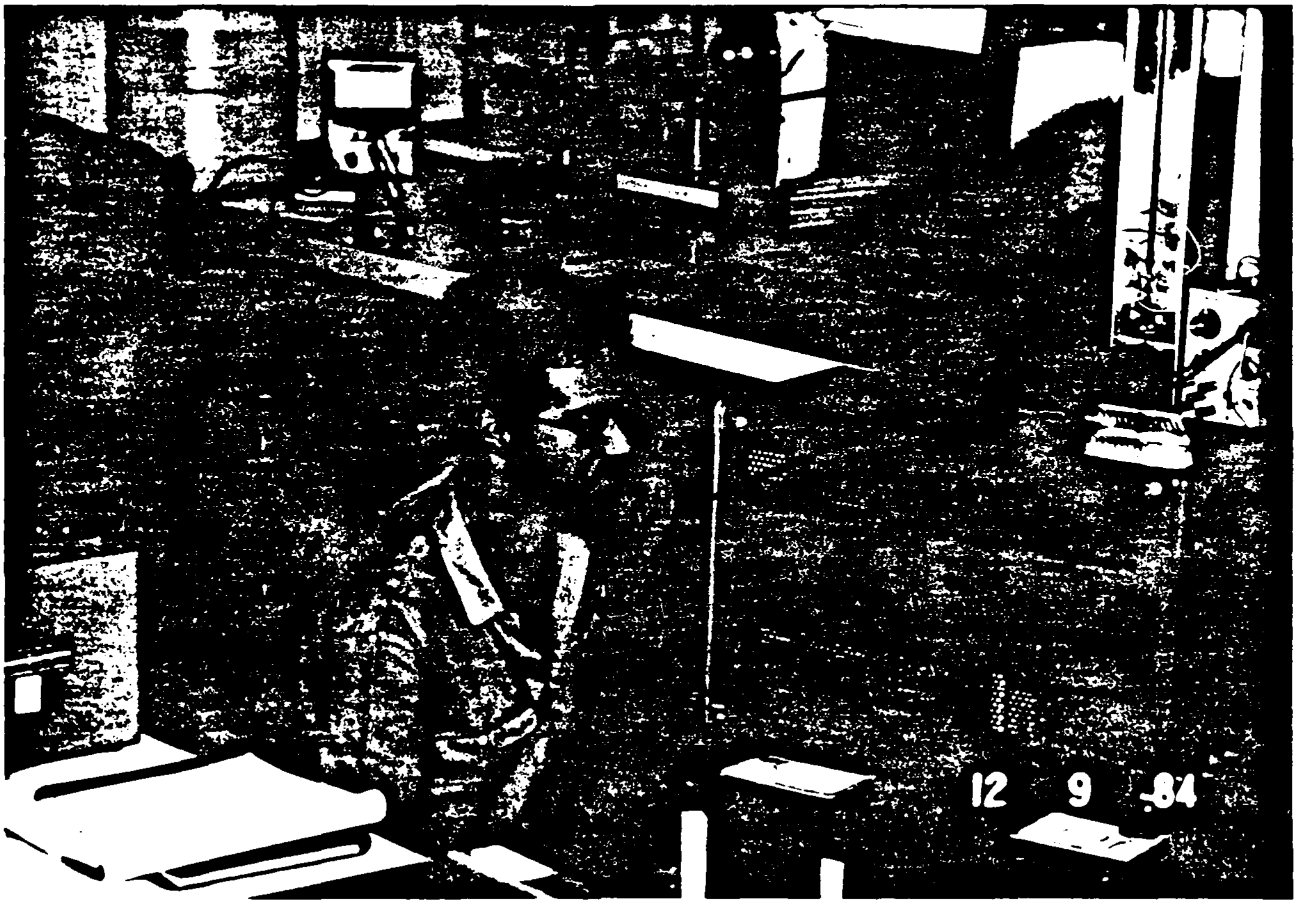


Figure 4. Alejandro Ubeda adjusting the amplitude of the pulse used to excite coils for exposure of eggs.

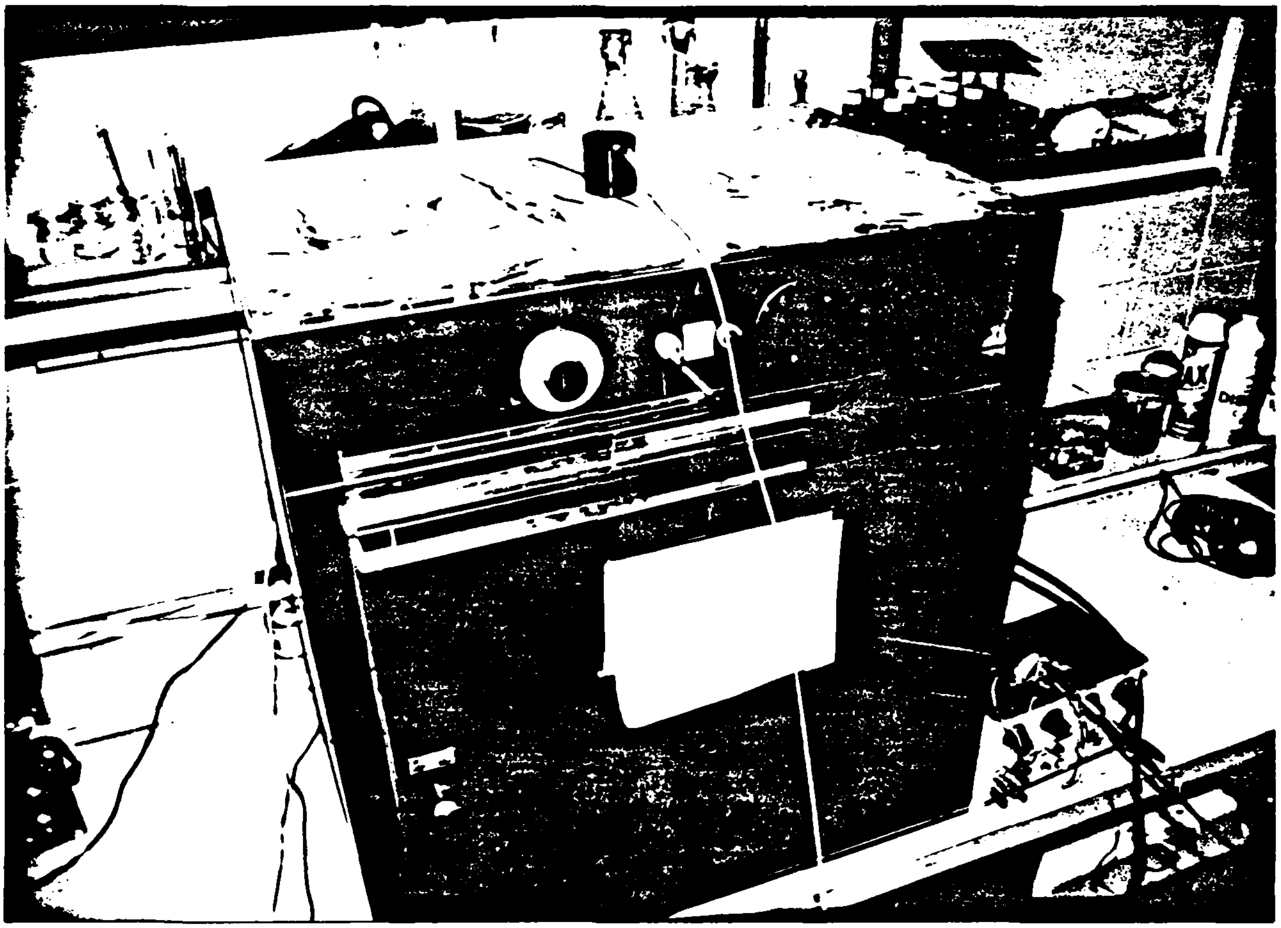


Figure 5. Photograph showing placement of accelerometer on large incubator for measurement of mechanical vibrations.

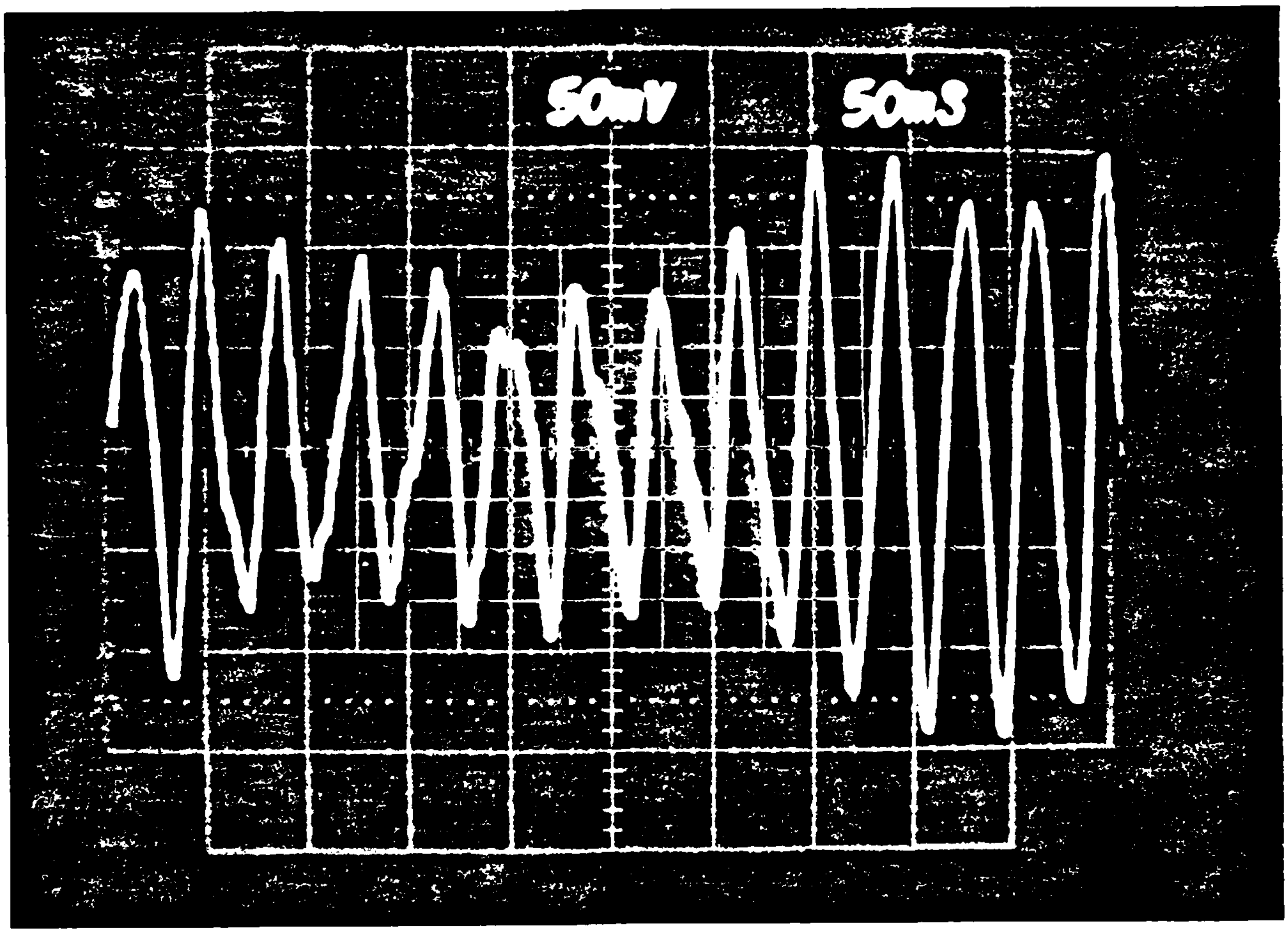


Figure 6. Oscilloscope photograph of output signal from accelerometer when placed on the larger incubator.

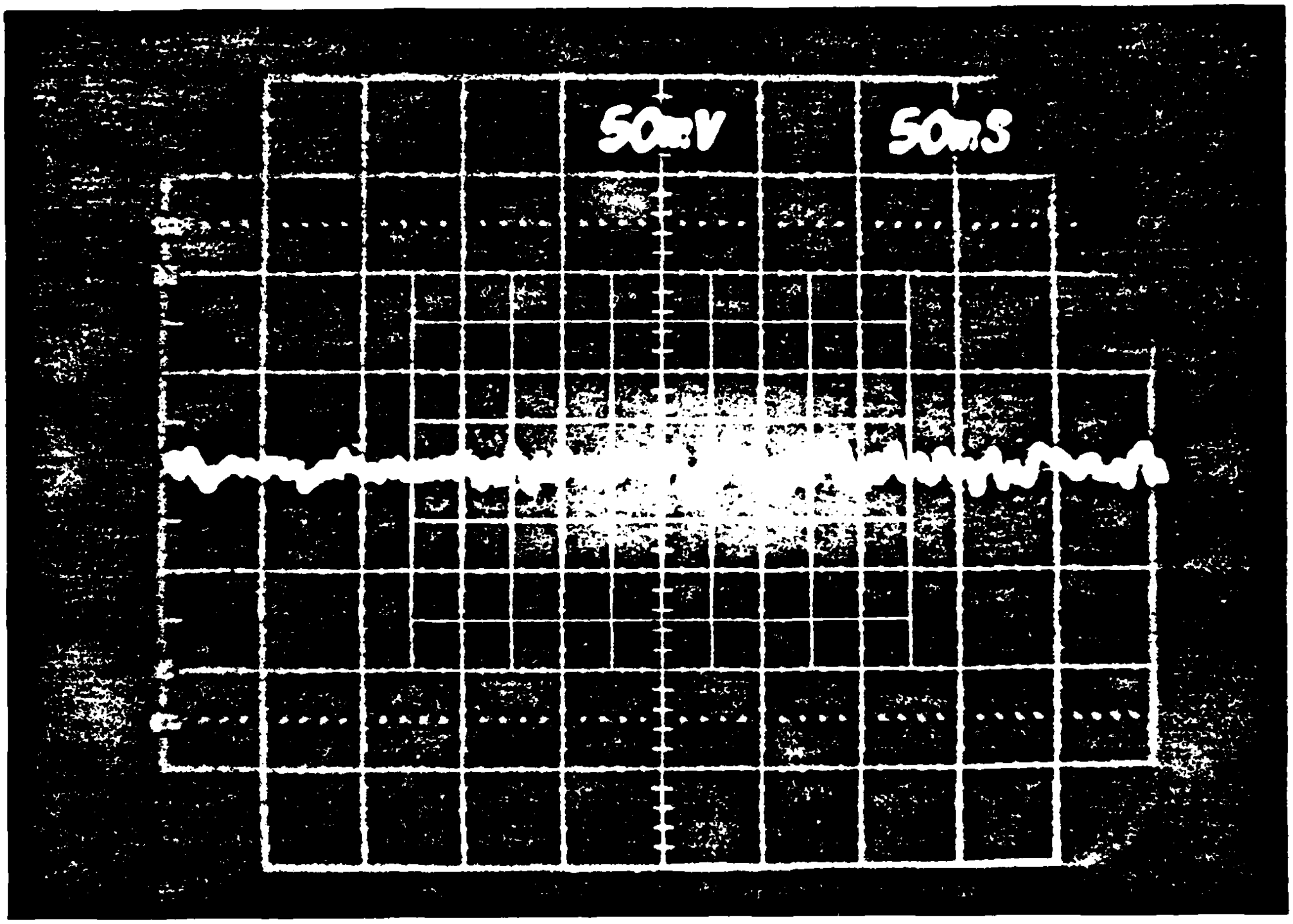


Figure 7. Oscilloscope photograph of output signal from accelerometer when placed on the smaller incubator.

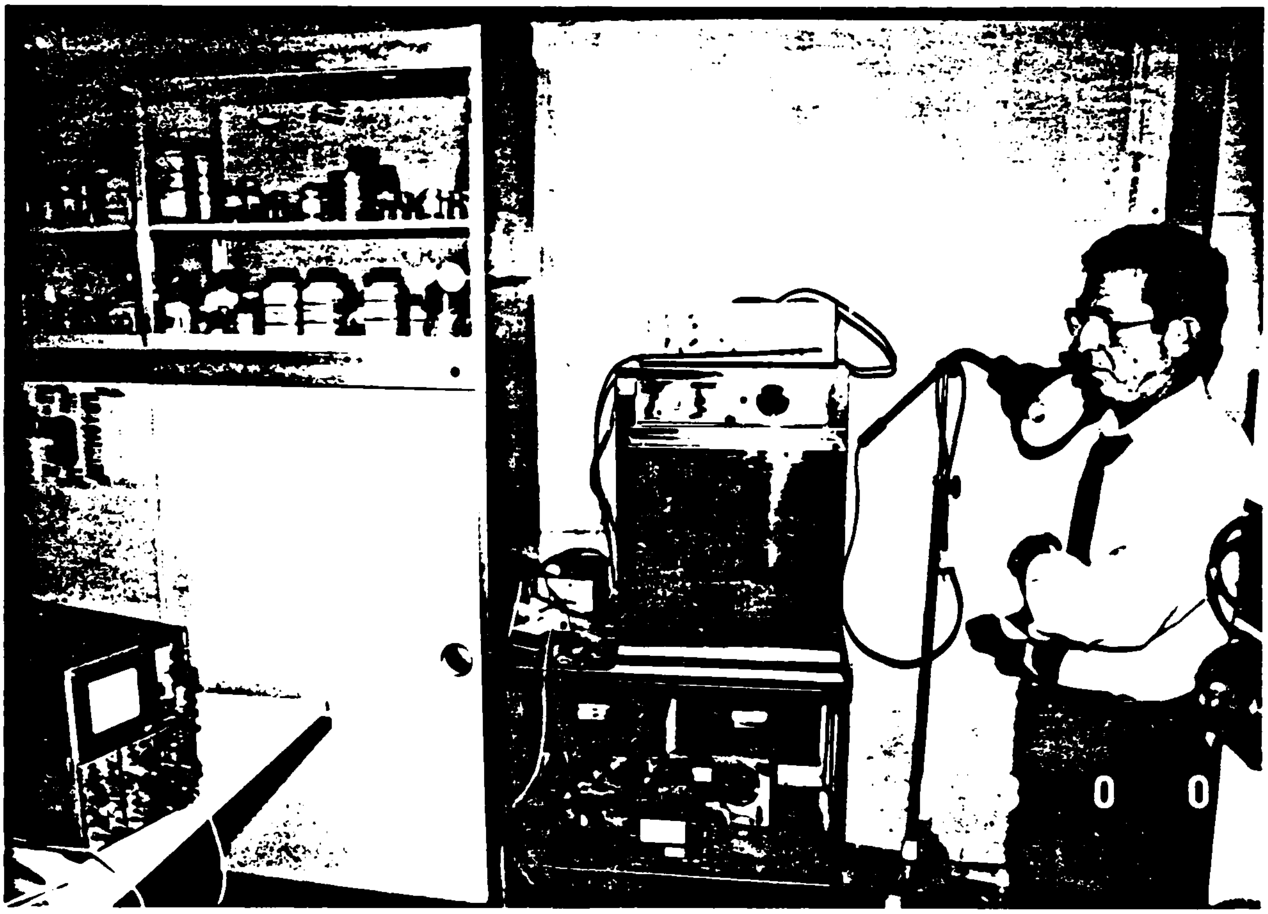


Figure 8. Smaller incubator showing placement of pulse generator on top.

Figure 9. Discussions between E. Berman and J. Leal.

Figure 10. Discussions between E. Berman and J. Leal.

ATTACHMENT A

CHECKED BOX APPLIES | X ORDER FOR SUPPLIES OR SERVICES | REQUEST FOR QUOTATIONS NO. EPA Ref: RW17930966-01-0

THIS IS NOT AN ORDER. See DD Form 1155c

1. Contract/Purch Order No.	2. Delivery Order No.	3. Date of Order	4. Requisition/Purch Request No.
	N00014-84-F-0167	84JUL18	NR 665-037/5-18-84 (441)

6. Issued by — Code N00014
Procuring Contracting Officer
Office of Naval Research
800 North Quincy Street
Arlington, Virginia 22217

7. Admin Stered by — Code N00014
Research Technology and International Branch, Code 512, Office of Naval Research, 800 North Quincy Street
Arlington, Virginia 22217

9. Contractor/Quoter — Name and Address
ENVIRONMENTAL PROTECTION AGENCY
Office of Radiation Programs (ANR 458)
401 M Street, S.W.
Washington, D.C. 20460
ATTN: Ray Brandwein

10. Deliver to FOB Point by: SEE BLOCK 19 BELOW

12. Discount Terms: N/A

13. Mail Invoices to: ONR, Code 512, See Page 2

14. Ship to — Code N00014
Associate Director for Life Sciences
Office of Naval Research, Code 440
800 North Quincy Street
Arlington, Virginia 22217

15. Payment will be made by — Code N00179
Commanding Officer
U.S. Navy Regional Finance Center
CM#3, Room 206, Attn: Code 40
Washington, D.C. 20371

MARK ALL PACKAGES AND PAPERS WITH CONTRACT OR ORDER NUMBER

DELIVERY — This delivery order is subject to instructions contained on this side of form only and is issued on another Government agency or in accordance with and subject to terms and conditions of above numbered contract.

PURCHASE — Reference your ___ furnish the following on terms specified herein, including for U.S. purchases General Provisions of Purchase Order on DD Form 1155r (EXCEPT CLAUSE NO. 11 APPLIES ONLY IF THIS BOX IS CHECKED AND NO. 15 IF THIS BOX IS CHECKED) special provisions and delivery as indicated. This purchase is negotiated under authority of 10 USC 2304(a)(3) or as specified in the schedule if within the U.S., its possessions or Puerto Rico; if otherwise, under 2304(a)(6).

If checked, Additional General Provisions apply. Supplier shall sign "Acceptance" on DD Form 1155r and return ___ copies.

ACCOUNTING AND APPROPRIATION DATA — ACCOUNTING CLASSIFICATION (REV 7-65)

Item	Appropriation Symbol and Subhead	Object Class	Bureau Cont. No.	Sub Allot	Auth. Acct'g Act'y	Trans Type	Property Acct'g Act'y	Coun try	Cost Code	Amount
AA	1741319.NRAE NR 665-037	000	RA441	0	068342	2B	000000		041010000A00	$3,687.00

Item No.	Schedule of Supplies/Services	Quantity Ordered/Accepted	Unit	Unit Price	Amount
0001	The purpose of this order is to provide support for two scientists to visit the laboratory of Professor Jose Delgado in Spain to evaluate and fully characterize the exposure apparatus he has used to expose chick embryos to pulsed magnetic fields. This support includes travel to Madrid, Spain from 10 to 17 September 1984 and per diem and air freight shipping for electronic test equipment and shall be conducted in accordance with Environmental Protection Agency letter dated 1 May 1984. See attachment for EPA approval.				$3,687.00

BLOCKS 26 THROUGH 42 ARE NOT APPLICABLE

*If quantity accepted by the Government is same as quantity ordered, indicate by ✓ mark. If different, enter actual quantity accepted below quantity ordered and encircle.

24. UNITED STATES OF AMERICA
20 JUL 1984
BY [signature]
JOSEPH C. ELY
CONTRACTING OFFICER

26. Quantity in column 20 has been: Inspected / Received / Accepted and conforms to the contract except as noted

Date — Signature of Authorized Government Representative

36. I certify this account is correct and proper for payment.

Date — Signature and Title of Certifying Officer

27. Ship No. — 28. D.O. Voucher No. — 30. Initials — Partial / Final — 32. Paid by — 33. Amount Verified Correct for — 31. Payment: Complete / Partial / Final — 34. Check Number — 35. Bill of Lading No.

37. Received at — 38. Received by — 39. Date Received — 40. Total Containers — 41. S/R Account Number — 42. S/R Voucher No.

STANDARD FORM 36, JULY 1966
GENERAL SERVICES ADMINISTRATION
FED. PROC. REG. (41 CFR) 1-16.101

CONTINUATION SHEET

REF. NO. OF DOC. BEING CONT'D.
EPA Ref: RW17930966-01-0
N00014-84-F-0167

NAME OF OFFEROR OR CONTRACTOR

ITEM NO.	SUPPLIES/SERVICES	QUANTITY	UNIT	UNIT PRICE	AMOUNT

BILLING AND PAYMENT

The total amount available under this Government Order is $3,687.00. Billing(s) should contain reference to this Government Order No. N00014-84-F-0167 and should be submitted to the Office of Naval Research, Attn: Code 512, 800 North Quincy Street, Arlington, Virginia 22217. The amount of $3,687.00 is to cover the period 18 July 1984 through 31 December 1984.

EPA reimbursable account numbers:

4X6C33D005	$2,200.00
4X6E61H015	$1,487.00

PERIOD OF PERFORMANCE

The performance of work hereunder shall commence on 18 July 1984 and shall be completed by 31 December 1984. Obligations under this Order shall not be incurred beyond the above date.

REPORTS

Periodic reports shall be submitted, as required, to the Scientific Officer specified in Block 14 on Page 1.

AUTHORITY

This Government Order is being negotiated pursuant to the provisions of Section 601 of the Economy Act of 30 June 1932, 47 Stat. 417, as amended, 31 U.S.C. 1535.

IAG Title: "Pulsed Magnetic Field Experiment Evaluation"

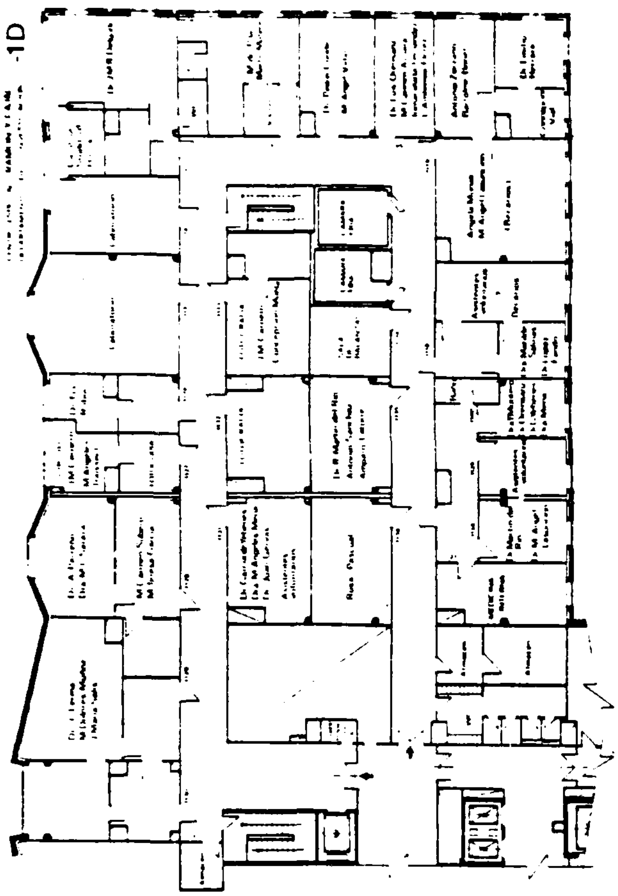
Navy Project Officer:

Dr. Michael Marron 696-4038
Office of Naval Research
800 North Quincy Street
Arlington, Virginia 22217

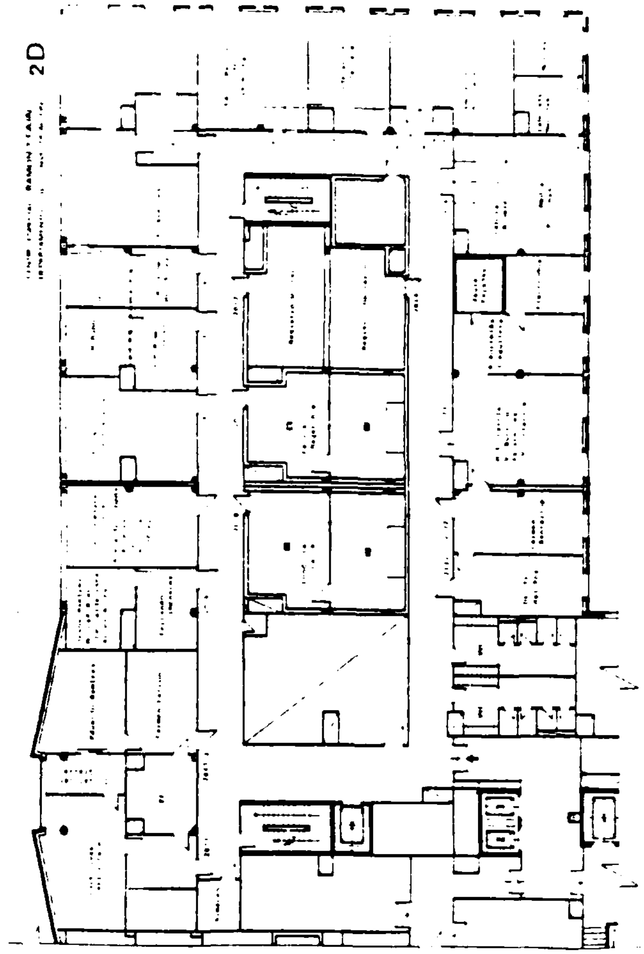
EPA Project Officer

Richard A. Tell FTS 545-2440
Office of Radiation Programs
Electromagnetic Radiation Analysis Branch
P. O. Box 15027
Las Vegas, Nevada 89114

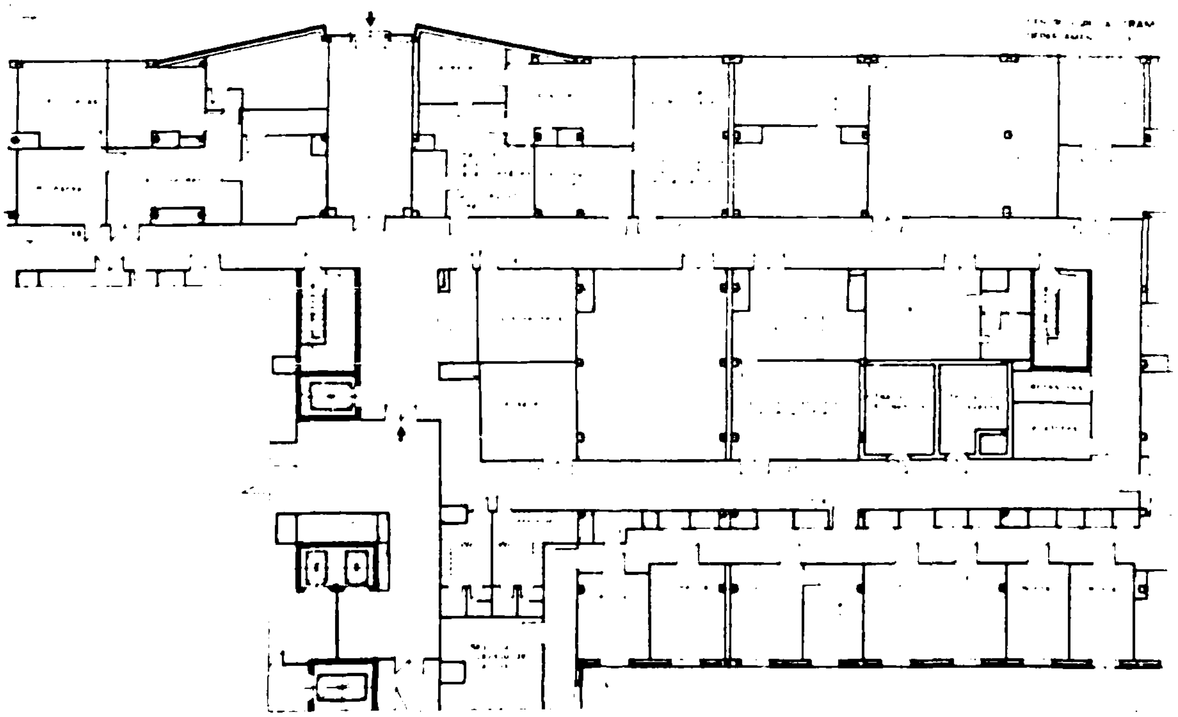
-1D



2D



-3D



ATTACHMENT C

MAGNETIC FIELDS IN BIOLOGY

José M.R. Delgado, M.D.

Centro Ramón y Cajal

Madrid 34. Spain

1984

INTRODUCTION

The beneficial effects of **MAGNETIC FIELDS** in the therapy of non-unions have been well documented in thousands of patients.

However, selection of the most effective parameters, including the intensity, frequency, shape, and duration of treatment require further experimentation.

We must learn more about the mechanisms of bone repair and bone growth; the magnetic flux through the heterogeneous medium of membranes, cells, and extracellular spaces; the intensity and distribution of local electrical currents generated by magnetic fields; and the short and long term biological reactivity of tissues.

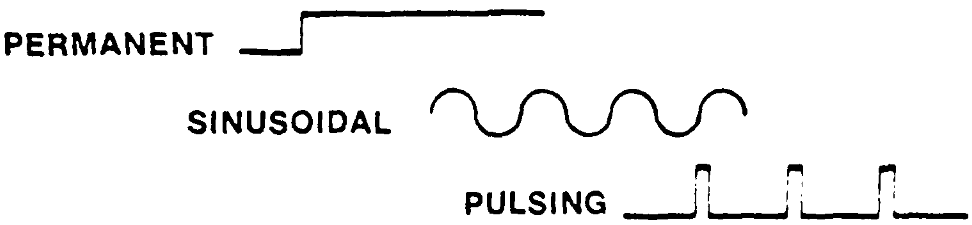
Specific electromagnetic applications have beneficial effects while others may produce unwanted results. A variety of biological processes can be influenced by electromagnetic fields including bone growth, genetics, embryogenesis, infections, nerve repair, and brain functions.

In order to contribute to this knowledge, our group at the Centro Ramón y Cajal in Madrid, Spain is conducting a multidisciplinary program in the following areas of **BIOELECTROMAGNETISM**: (1) Basic Research; (2) Development of new **EMF** instrumentation; (3) Standard therapy of non-unions; (4) Clinical research in traumatology, surgery, and neurology; (5) Preventive medicine; and (6) Evaluation of artificial contamination of the environment with possible medical and ecological consequences.

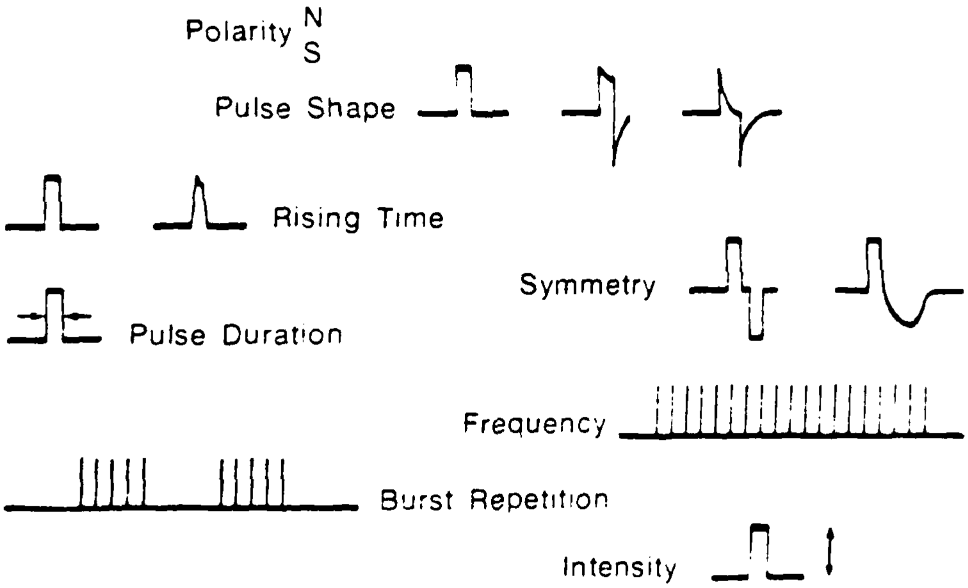
A brief summary of results is presented here. For more details, see our selected publications listed on the last page.

MAGNETIC FIELDS

Magnetic Fields may be:



Most biological and medical effects have been obtained with pulsing magnetic fields and the results are related to the following **PARAMETERS**:



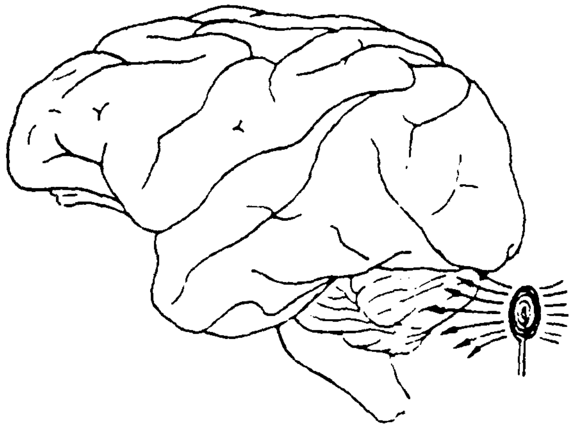
Daily Duration (10 h day: 10pm-8am)
Total Duration (1.200 h in 4 months)

Characteristics of Parameters depend on:

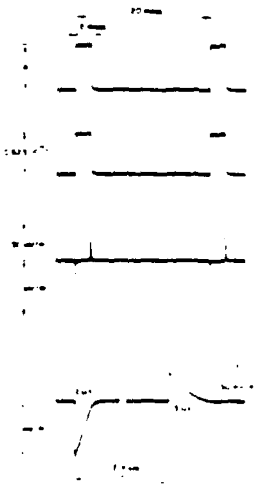
GENERATOR: Construction and setting
COIL: Turns, diameter, electric constants
TISSUE: Heterogeneity (Impedance)
DISTANCE from coils

CHOICE of Therapeutic Parameters should be based on.
ANIMAL EXPERIMENTATION
CLINICAL DATA

MAGNETIC AND ELECTRIC FIELDS



Intensity of **EMF** decreases exponentially with distance from the coil.



A: Current at the coil.

B: Magnetic flux density at 10 mm.

C: Induced electrical field

D: Induced electrical field wave follows the first derivative of the driving current

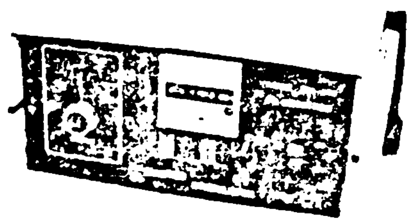
Extracellular fluids have uniform conductivity. Cell membranes have a high, heterogeneous impedance. Transmembrane aminosugar strands may detect and amplify weak fields, triggering powerful intracellular responses. Parametric windows, cooperative processes, and resonant effects are possible.

NEW EMF INSTRUMENTATION

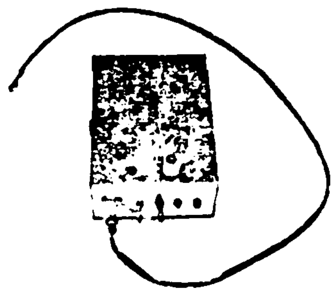
Biomagnetic research requires new instrumentation for the **generation** of signals with adjustable parameters, for the **microminiaturization** of stimulators, and for the **detection** of fields in tissues and in the environment

Cerebellar **EMF** stimulator

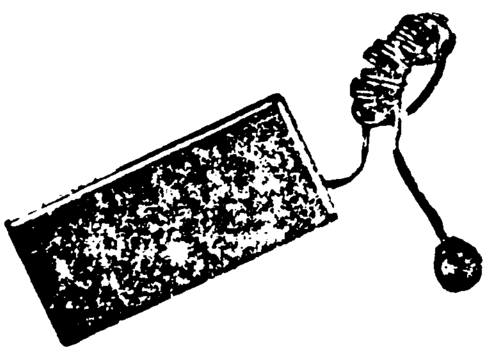
High Voltage stimulator



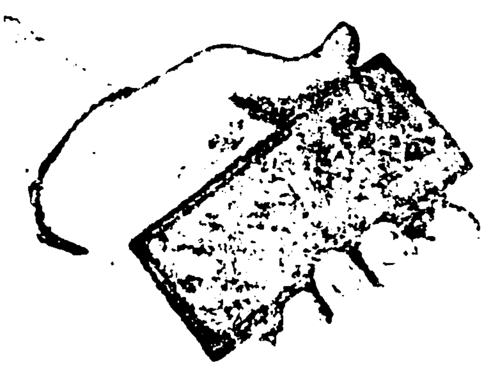
EMF generator



EMF detector



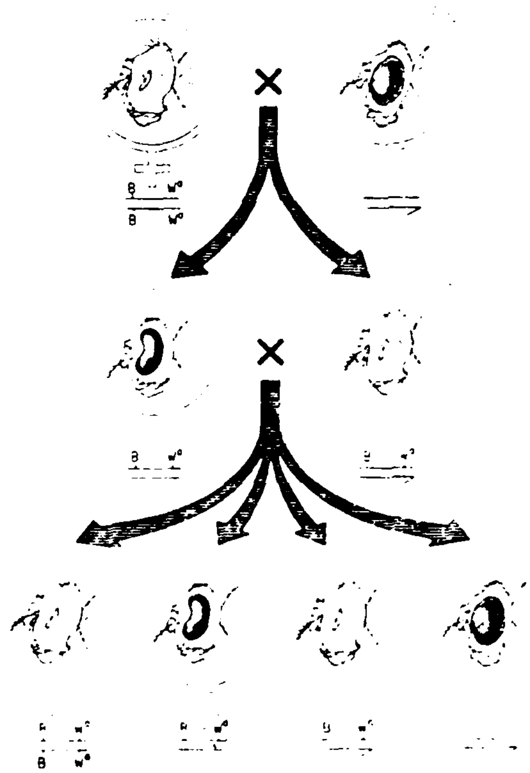
Mini **EMF** generator



Cumulative **EMF** detector

GENETIC INFLUENCE OF EMF

Drosophila melanogaster males were exposed for 70 hours to pulsated **EMF** with parameters similar to those used for therapy of non-unions. The standard Basc test was performed to determine whether production of sex-linked recessive lethals in mature sperm was modified by **EMF.**

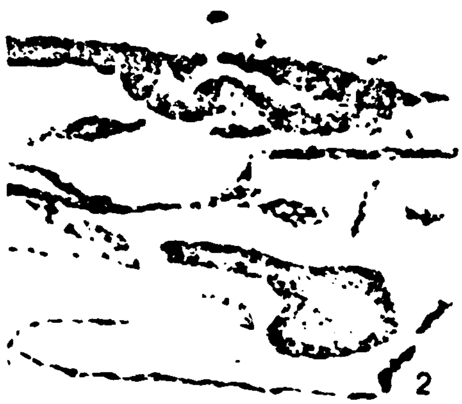


In this test, a lethal in the treated X-chromosome is detected by the absence of wild-type round red eyed males from an F_2 culture. The study of 6,000 X-chromosomes indicates that **EMF** exposure has a mild mutagenic effect.

CELLULAR AND EMBRYOLOGICAL EFFECTS

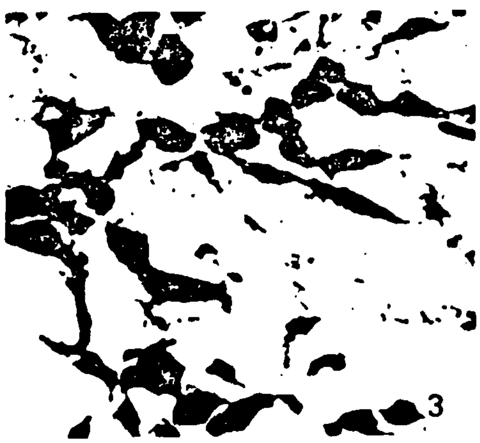
Acceleration for bone growth by **EMF** may be related to modifications in cellular activities and changes in the local electrical and chemical environment.

One model for investigation of embryological and cellular effects is the developing chick egg (1). 100 Hz, 1.2 μTesla produced powerful inhibition of embryogenesis (2). Different organs in the embryo had specific parametric sensitivity and their development could be accelerated or retarded. Cell mitosis was considerably modified by **EMF**. Glycosaminoglycanes (3), which are essential in cellular activities including migration, seemed specially reactive to **EMF** (4).



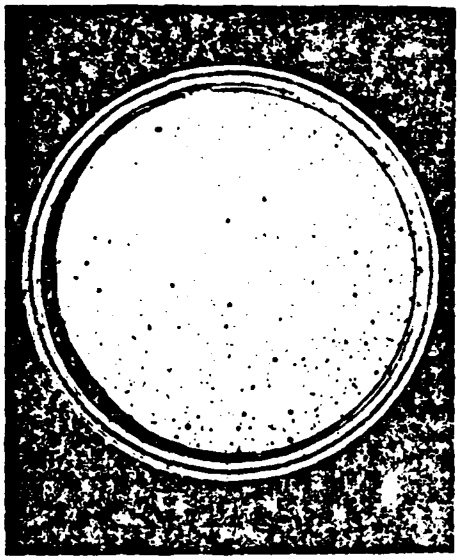
CONTROL

EMF

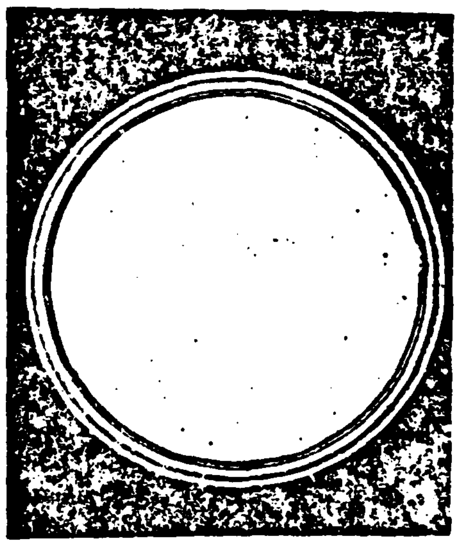


MICROBIOLOGY: INFECTIONS

Over one hundred colonies of Gram negative bacteria (Lactobacillus acidophilus) were cultured at 38° C in Petri dishes for 72 hours. The inoculum (initial no. of bacteria per ml) was 24.10³.



CONTROL



EMF Treated

Pulsed magnetic fields covering the parametric range of fields used in non-union therapy (0-40 Gauss) caused a 40 % reduction in the number of Lactobacillus colonies.

RAT MODEL TO STUDY EMF AND BONE REPAIR

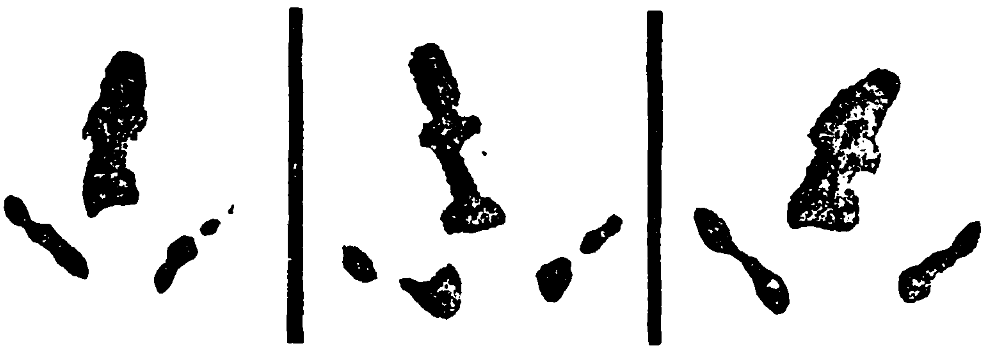
Rats with both hindlegs fractured are placed in a specially designed restraining unit with stretching pins to immobilize both legs in a therapeutic position and wheels to permit mobility.

One leg is treated with **EMF** (10 Hz. 20 Gauss) while the other leg remains as control. After 4 to 6 weeks there is evidence of improved bone healing in the treated leg. as shown by injection of the tracer 99m Tc methylene diphosphonate.

RAT IN THE PLASTIC RESTRAINER

The model is efficient. reliable. inexpensive. and easy to use.

Animals have tolerated these procedures well and remain in good health after over 1 year.



99m Tc **RATIO: TREATED / CONTROL**

2 weeks	4 weeks	6 weeks
0.36	1.29	1.51

THERAPY OF NON-UNIONS

Successful electromagnetic therapy of non-unions is well documented. One case, treated in Madrid by Dr. J. Palacios and his group, is shown here.

Patient **JMG,** 21 years old, leg fractured in accident August, 1980. Non consolidation after 10.5 months with casts. **EMF therapy:** 92 days (1,317 hours). Excellent result. Follow-up, 1983: Patient continues walking normally.

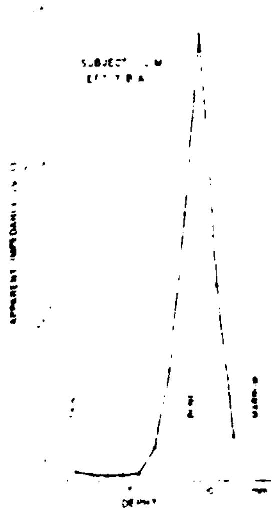
FUTURE TRENDS IN EMF THERAPY

1. Use of portable stimulators.
2. Increased rising time of pulses to obtain higher local electrical fields.
3. Determination of local impedance for greater effectiveness in therapy.
4. Follow-up results using non-invasive bone tomography.
5. Parametric adjustments to patient's condition including possible infections.
6. Timing of application considering rebound and local reactions.
7. Use in other orthopaedic problems, including fresh fractures, osteoporosis, and osteomyelitis.

IMPEDANCE TOMOGRAPHY OF BONES

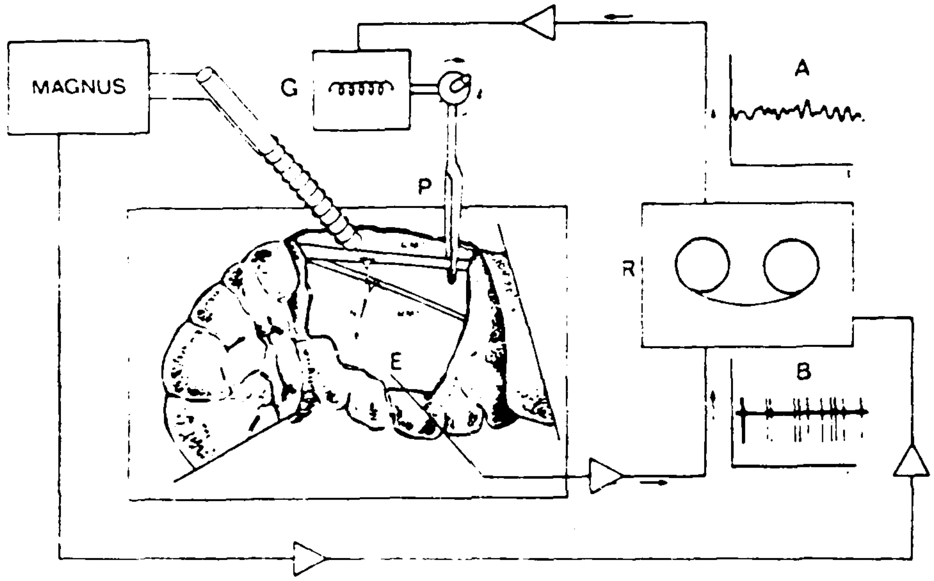
Bone and soft tissue impedance are modified by physiological and pathological situations. Their measurement is important in diagnosis and therapeutic follow-up including the evaluation of **EMF** treatment.

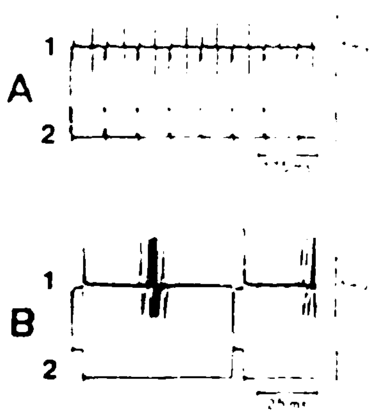
An array of 24 gold plated electrodes is placed over the skin of a superficial bone. Each electrode is driven sequentially by electrical pulses. Voltages measured at separate points reflect the conductivity of tissues crossed by the paths of current. Data processing by a microcomputer provides information on local resistivity and thickness of soft tissues, periosteum, bone, and bone marrow. The procedure is harmless, fast, and accurate.



NEURONAL ACTIVITY IN THE CRAB

Unitary activity of the tonic stretch receptor of the river crab was recorded in the dorsal nerve by means of microelectrodes. The signals were processed to obtain histograms of first order intervals, autocorrelation, and crosscorrelation.

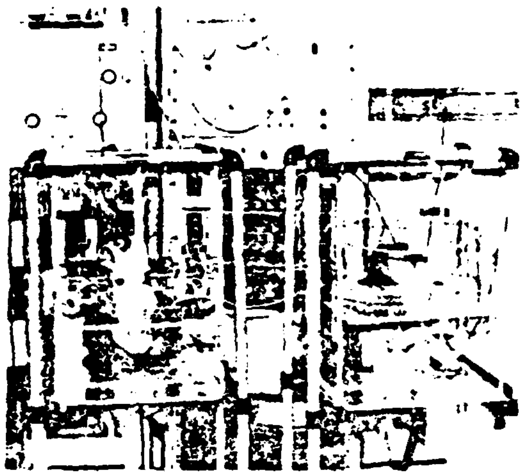




Results show that the receptor has considerable sensitivity to magnetic fields at intensities below 100 μT

At specific frequencies, there was synchronization between neuronal discharges and pulsated magnetic fields.

BRAIN AND BEHAVIOR EFFECTS



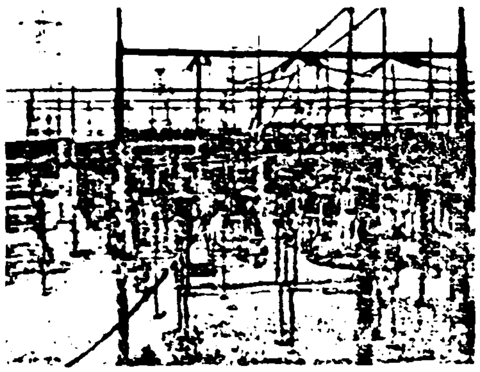
In monkeys, external application of pulsed magnetic fields (square, 1 msec, 50 Hz, 1-2 Gauss) beamed at the cerebellum for 9 hours, modified brain excitability.

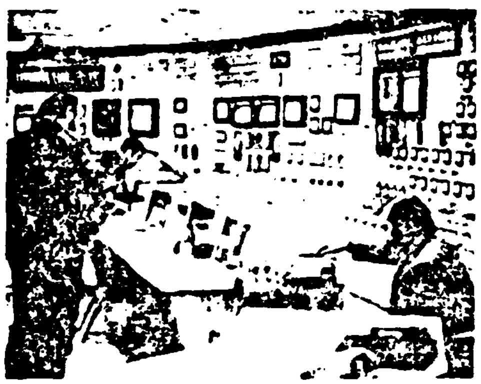
Thresholds were increased in the hippocampus and caudate nucleus; they decreased in the motor cortex and internal capsule; and no detectable effects were produced in other areas of the brain.

Using external coils around the head, 50 Hz produced relaxation and sleepiness with decreased mobility. In contrast, 100 Hz evoked restlessness and attempts to escape.

Electromagnetic non-invasive stimulation of the cerebral nervous system offers a new, powerful technology for the investigation and modification of brain functions

MAGNETIC CONTAMINATION OF OUR ENVIRONMENT





In cities, electromagnetic fields have increased exponentially during the last 50 years due to the development of electric power plants, power lines, industries, communications and household appliances.

High power lines in Spain emit tolerable **EMF** levels below 0.5 Gauss. Home appliances and electrical instruments may emit up to 25 Gauss or more.

Research is urgently needed in order to measure existing values, to establish tolerable doses, and to organize preventive medicine.

PARTICIPANTS IN THE PROJECT

José Luis MONTEAGUDO

José PALACIOS

Enrique MALBOYSSON

. . . .

Julián BUSTAMANTE	Rafael L. PORTOLES
José M. CARNERO	Ovidio MAROTO
Angel CRESPO	Juan C. MEDRANO
Caroline DELGADO	Antonio PARREÑO
Manuel Ga. GRACIA	Eduardo RAMIREZ
Claudio HERNANDEZ ROS	Luis RIVAS
María A. JIMENEZ	Maria L. SARAZA
Pablo JORGE	María A. TRILLO
Jocelyne LEAL	Alejandro UBEDA

END

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